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Serum Levels of Soluble Intercellular Adhesion Molecule-1 (ICAM-1) and the Expression of ICAM-1 mRNA in Uterine Cervical Cancer

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The purpose of this investigation was to evaluate the serum level of soluble intercellular adhesion molecule-1 (sICAM-1) in patients with squamous cell carcinoma of the cervix. Serum levels of sICAM-1 were measured by enzyme-linked immunosorbent assay in patients with cervical cancer (stage 0 through IV). Expression of mRNA in tumor tissues was evaluated by reverse transcriptase–polymerase chain reaction. Serum level of sICAM-1 in patients with advanced-stage (II–IV) or recurrent cervical cancer was found to be increased significantly. Expression of ICAM-1 mRNA was observed in most tumor tissues. Results suggest that sICAM-1 is shed from the cancerous tissue in patients with squamous cell carcinoma of the cervix.

Cell–matrix interactions are essential for the adhesion and migration of cells, wound healing, cell differentiation, and polarity [1, 2]. These interactions are mediated by the adhesion of cell-surface receptors, particularly of the integrins, to adhesive proteins [2, 3].

Intercellular adhesion molecule-1 (ICAM-1, CD54), a member of the immunoglobulin superfamily of adhesion receptors, has five extracellular immunoglobulin-like domains, a single transmembrane region, and a short cytoplasmic tail [4, 5]. ICAM-1 is expressed on various cell types, including fibroblasts, keratinocytes, vascular endothelial cells, macrophages, and glandular epithelial cells and stromal cells in the uterus [2, 6, 7]. ICAM-1 is a ligand for lymphocyte function-associated antigen-1 (LFA-1 or CD11a/CD18), which is present on lymphocytes, monocytes, and neutrophils [8].

ICAM-1 is an important early marker of immune activation and response [9–11]. The upregulated expression of ICAM-1 on cell surfaces is observed in a variety of diseases, including patients with rejection of liver allograft [9], autoimmune thyroiditis [10], Sjögren's syndrome [12], asthma [13], cutaneous T-cell lymphoma [14], melanoma [15], prostate cancer [16], ovarian cancer [17], and colonic cancer [18]. Coleman *et al.* [19] demonstrated the expression of ICAM-1 protein in high-grade intraepithelial squamous neoplasia of the cervix by immunohistochemistry and suggested that the expression was related to human papillomavirus infection.

Seth *et al.* [20] used a chemiluminescence technique to detect soluble ICAM-1 (sICAM-1) in the serum obtained from healthy young volunteers. They found that ICAM-1 existed in a membranous and a soluble form that lacks the cytoplasmic tail and the transmembrane region. Although its functional characteristics have not been elucidated, Rothlein *et al.* [21] suggested that the circulating molecule reflects inflammation, tissue damage, and proteolysis. When increased sICAM-1 binds LFA-1, which is expressed on lymphocytes, the lymphocytes cannot bind other cells via adhesion molecules, resulting in the inhibition of lymphocyte function [22].

In the present study, we measured serum levels of sICAM-1 in patients with squamous cell carcinoma of the cervix, and investigated the expression of ICAM-1 in tumor tissues by reverse transcriptase–polymerase chain reaction (RT–PCR).

MATERIALS AND METHODS

ELISA for detection of sICAM-1. We obtained 6-ml serum samples from 34 Japanese women with squamous cell carcinoma of the cervix before the initial treatment (9 in stage 0, 7 in stage I, 3 in stage II, 5 in stage III, and 3 in stage IV), 7 patients with a recurrence of this cancer, and 16 healthy volunteers. Venous blood was allowed to clot at 4°C and was then centrifuged at 1000*g* for 15 min to remove the clot. Each serum sample was divided into 1-ml aliquots and frozen at −70°C until assayed.

Serum levels of sICAM-1 were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) through use of an ICAM-1 test kit (T Cell Diagnostics, Cambridge, MA). The serum was allowed to thaw at room temperature and was then diluted 100-fold in a dilution buffer (T Cell Diag-

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nostics). The assay was performed in duplicate as follows: standards or diluted samples (25 μl) were placed in polystyrene microtiter wells that were precoated with a mouse anti-human ICAM-1 monoclonal antibody. A horseradish peroxidase-conjugated mouse anti-human ICAM-1 monoclonal antibody was immediately added to the wells, which were then incubated for 2 hr at room temperature. After the wells had been washed with phosphate-buffered saline (PBS, T Cell Diagnostics), a chromogen solution (T Cell Diagnostics) was added. The reaction was terminated by the addition of 2 *N* H_2SO_4 (Wako, Osaka, Japan) and absorbance was measured at 490 nm by use of a microplate reader (Model 3550, Bio-Rad Laboratories, Richmond, CA). All the reactions were performed at room temperature.

Data are the mean ± SD. Difference were evaluated by Scheffe's *F* test using StatView 4.01 (Abacus Concepts, Berkeley, CA). A value of $P < 0.05$ was regarded as statistically significant.

RT–PCR for detection of ICAM-1 mRNA. Samples (100-mg) of cervical squamous cell carcinoma tissues were obtained from seven patients in an advanced stage (stage III) of the disease. As controls, we obtained specimens of normal cervical tissue from four women who had undergone hysterectomy for the evaluation of a benign disease. As positive controls, we used myelomonocytic cell line, HL-60, on which ICAM-1 expression had previously demonstrated [23, 24]. Each tissue sample was soaked in 1 ml of Isogen solution (Nippon Gene, Tokyo, Japan), homogenized by use of a Polytron (Type PT 10/35, Kinmatica GmbH, Luzern, Switzerland), stored at room temperature for 5 min, and shaken vigorously for 15 sec after the addition of 0.2 ml of chloroform. The homogenates were centrifuged at 12.000*g* at 4°C for 15 min and then 0.5 ml of isopropanol was added to the aqueous phase. Each aliquot was stored at room temperature for 10 min and centrifuged at 12,000*g* at 4°C for 10 min. To the precipitate, 1 ml of 75% ethanol (Wako) was added. The aliquot was shaken vigorously and centrifuged at 12.000*g* at 4°C for 15 min. The precipitate was then dried briefly and dissolved in water.

ICAM-1 gene expression was analyzed by the RT–PCR method through use of an RNA PCR Kit with AMV RTase (Takara, Tokyo, Japan). RNA was reverse-transcribed into complementary DNA (cDNA). In brief, the reaction mixture (5 m*M* $MgCl_2$, 1× RNA PCR buffer [10 m*M* Tris–HCl, 50 m*M* KCl, pH 8.3], 1 m*M* dNTP mixture [dATP, dCTP, dGTP, dTTP] [Takara], RNase Inhibitor, 1 U/μl [Takara], reverse transcriptase, 0.25 U/μl [Takara], and 2.5 μM random 9 mer [Takara]) was added to the RNA solution containing 1 μg of total RNA, and the mixture was incubated at 30°C for 10 min. After incubation at 50°C for 25 min, the mixture was heated to 99°C for 5 min and then chilled on ice.

To perform the PCR assay, primer sets for ICAM-1 (upper primer: 5'-GTCCCCCTCAAAAGTCATCCTG-3' and lower primer: 5'-CCCTCGTCCTCTGCGGTCACAC-3') were designed according to the previously reported sequence [23, 24], synthesized by the phosphoramide method on a DNA synthesizer (Model 8700, Biosearch, San Rafael, CA), and purified on Sephadex G50 columns (Pharmacia LKB Biotechnology, Uppsala, Sweden) and by high-performance liquid chromatography (HPLC). Forward and reverse primers were located within the open reading frame; the predicted size of the PCR product was 759 bp. The size of the RT–PCR product is apparently different from that of the PCR product of genomic DNA.

The cDNA transcribed from 1 μg of total RNA was amplified on a thermal cycler (Model PJ2000, Perkin Elmer, Norwalk, CT) in a total volume of 80 μl containing 4 m*M* $MgCl_2$, 1× RNA PCR buffer, 0.2 μM of each primer, and 2.5 units of *Taq* DNA polymerase (Takara). PCR was carried out for 40 cycles with thermal cycler conditions for denaturing at 94°C for 3 min, annealing at 59°C for 2 min, and extension at 72°C for 2 min. The PCR products were separated by 1.2% agarose gel (Takara) electrophoresis and visualized by ethidium bromide (Takara) staining.

RESULTS

Serum levels of sICAM-1. Serum levels of sICAM-1 in healthy volunteers, in patients with cervical cancer in stages 0, I, II, III, and IV and in patients with recurrent tumor were 294 ± 84, 274 ± 39, 339 ± 136, 554 ± 131, 498 ± 155, 451 ± 108, and 503 ± 111 ng/ml, respectively. Although the serum level of sICAM-1 in patients with early-stage cervical cancer (stage 0–I) did not increase, serum levels of ICAM-1 in patients with advanced-stage cervical cancer or recurrent cancer were significantly increased (control vs stage II, $P < 0.05$; control vs stage III, $P < 0.05$; control vs recurrence, $P < 0.01$; stage 0 vs stage II, $P < 0.025$; stage 0 vs stage III, $P < 0.05$; stage 0 vs recurrence, $P < 0.025$; Scheffe's *F* test) (Table 1).

Expression of ICAM-1 mRNA. As shown in Fig. 1, ICAM-1 mRNA expression had been observed in HL-60 cells as previously described [23, 24]. The expression of ICAM-1 mRNA was observed in six of seven tumor tissues. ICAM-1 mRNA expression in four samples of normal cervical tissue was below the detection level.

DISCUSSION

ICAM-1 expression has been observed on various cell types in normal, inflammatory, and tumor tissues, including fibroblasts, keratinocytes, vascular endothelial cells, lymphocytes, monocytes, glandular epithelial cells and stromal cells in the uterus, carcinomas of the gastrointestinal tract

TABLE 1

Serum Levels of sICAM-1 in Healthy Volunteers, in Patients with Cervical Cancer in Stages 0, I, II, III, and IV, and in Patients with Recurrent Tumor

Group	Number of patients	Serum sICAM-1 (ng/ml)[a]
Control	16	249 ± 84
Stage 0	9	274 ± 39
Stage I	7	339 ± 136
Stage II	3	554 ± 131*†
Stage III	5	498 ± 155*††
Stage IV	3	451 ± 108
Recurrence	7	503 ± 111**†

[a] Data are the mean ± SD.

* $P < 0.05$, ** $P < 0.01$, vs control, Scheffe's F test.

† $P < 0.025$, †† $P < 0.05$, vs stage 0, Scheffe's F test.

[18, 25], ovarian cancer [17], and malignant melanoma [2, 6, 7, 26].

The involvement of ICAM-1 in the pathogenesis of malignant tumors has been examined, especially in malignant melanoma. Reports by Johnson *et al.* [27], Natali *et al.* [15], and Kageshita *et al.* [28] suggest that increased expression of ICAM-1 on melanoma cells may be positively correlated with a greater risk of metastasis. In contrast, evidence from Vanky *et al.* [29] suggests that expression of ICAM-1 on tumor cells may facilitate recognition by autologous lymphocytes and thus reduce metastasis. Harning *et al.* [26] observed increased serum levels of sICAM-1 in patients with malignant melanoma and found a correlation between increasing serum level of sICAM-1 and prognosis. ICAM-1 is also expressed on keratinocytes and blood vessels in benign inflammatory disorders, such as dermatitis and allergic eczema, and this expression appears to be correlated with the extent of disease [14].

Tsujisaki *et al.* [25] used immunochemical methods to detect cell surface and sICAM-1 in cell lines obtained from colonic carcinoma, gastric carcinoma, pancreatic carcinoma, hepatocellular carcinoma, and lung adenocarcinoma. They also reported that serum levels of sICAM-1 were elevated in patients with gastric cancer, colonic cancer, gall bladder cancer, pancreatic cancer, and esophageal cancer, especially in patients with liver metastasis. They suggested that sICAM-1 may block the attachment of cytotoxic T cells and/or NK cells to cancer cells, since LFA-1 could be blocked with sICAM-1, and that the higher levels of serum sICAM-1 in malignant diseases may reflect the host's immune response to malignant cells and surrounding cells. Harning *et al.* [26] suggested that the elevated serum levels of ICAM-1 observed in patients with malignant melanoma are indicative of an enhanced host cell-mediated immune response to a primary tumor or may represent ICAM-1 shed *in situ* by melanoma cells. Burrows *et al.* [30] maintained that ICAM-1 expression in most carcinomas is a passive event resulting from induction by infiltrating leukocytes and is unrelated to the tumor cells themselves. Yamamoto *et al.* [31] reported that peripheral blood mononuclear cells from cancer patients revealed an increase of ICAM-1 expression.

Coleman and Stanley [32] reported that the expressions of ICAM-1, vascular cellular adhesion molecule-1 (VCAM-1), and E-selectin were significantly up-regulated in the high-grade intraepithelial squamous neoplasia of the cervix using immunohistochemical method. ICAM-1 expression was observed in vessels, keratinocytes, and mononuclear cells in these lesions. They suggested that the enhanced expression of these adhesion molecules may be functionally important in enabling the local recruitment of immunocompetent cells.

Rothlein *et al.* [21] suggested that sICAM-1 serves as a marker of inflammation, tissue damage, and proteolysis. The source and function of sICAM-1 in humans remain unclear. Cardiac transplant rejection is associated with an increase in the level of sICAM-1, a finding that suggests an increase in sICAM-1 may indicate activation of cells that participate in allograft rejection [33]. sICAM-1 is also be detected in cell culture supernatants of normal lymphocytes and established cancer cell lines [25, 34].

ICAM-1 expression in tissues is induced by various inflammatory cytokines, such as IL-1, TNF-α, and IFN-γ [6, 35]. These cytokines, which are produced by various cells, including inflammatory cells and tumor cells, sometimes upregulate cellular binding between tumor cells and endothelial cells through the induction of adhesion molecules [30] and increase the incidence of metastasis [36, 37].

In the present study, we showed that the serum level of sICAM-1 is significantly elevated in patients with advanced cervical squamous cell carcinoma and that ICAM-1 mRNA is expressed in tumor tissues. Therefore, an increase in the serum levels of sICAM-1 may reflect the enhanced expression of the molecule in the cancerous tissues. This increase in production is detected using ELISA perhaps due to the fact that the tumor reaches a certain size or spreads beyond the uterus (i.e., higher than or equal to stage II). Although sICAM-1 level of patients with stage IV disease did not have a significant difference compared to the patients with earlier stages, we think that the number of the patients with stage IV disease is too small to make a statistical analysis. Inflammatory cells may infiltrate the tumor tissue partly by

FIG. 1. Electrophoresis of RT–PCR products. Representative results are shown. Lane 1, normal cervical tissue; lanes 2–5, squamous cell carcinoma of the cervix (stage III); lane 6, HL-60 cells.

interaction of their membranous LFA-1 and ICAM-1 on endothelial cells. We hypothesize that sICAM-1 is produced mainly by carcinoma cells and that sICAM-1 suppresses the infiltration of inflammatory cells by a competitive inhibition of the membranous ICAM-1/LFA-1 interaction. Our results suggest that ICAM-1 is present in squamous cell carcinoma of the cervix.

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